51. Phytochromes in widespread photosynthetic algae reveal origins of plant signaling proteins

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Project Goals: To develop a systems biology approach to the study of the widespread marine alga *Micromonas* and use it to investigate gene function, pathways and consequences of environmental perturbations on primary production.

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Phytochromes are photoswitches that play master regulatory roles in land plants, fungi, and bacteria. In land plants, phytochromes have undergone gene family expansion, and now perform distinct and overlapping photoregulatory functions to optimize photosynthesis or to initiate flowering and seed dispersal. Light sensing by phytochrome relies on a covalently bound linear tetrapyrrole (bilin) chromophore, whose photoisomerization triggers a reversible photoconversion between red and far-red absorbing states that modulates downstream signaling events. Phytochrome involves a vast and complicated network of genes to control developmental transitions in land plants. Although widespread in bacteria, the limited distribution of eukaryotic phytochromes is an obstacle to creating plausible evolutionary scenarios and understanding of early functional roles. Fungi and some heterokont algae possess phytochromes but other unicellular eukaryotes with sequenced genomes do not, such as most Archaeplastida lineages, including model green algal species (e.g. the chlorophytes *Chlamydomonas reinhardtii* and *Chlorella vulgaris*).

We identified phytochrome-related sequences in the genome of the prasinophyte alga *Micromonas pusilla* CCMP1545. The *M. pusilla* phytochrome gene model was confirmed using multiple lines of evidence, including immunoblot and proteomics analyses. Phylogenetic analyses and the common protein domain architecture in prasinophyte and plant phytochromes (except for a C-terminal response regulator domain in prasinophytes), support the idea of a shared ancestry as a green phytochrome lineage. We then investigated the expression of phytochrome and genes in pathways known to be influenced by phytochrome activity over the day:night cycle in *M. pusilla* by using directional pair end RNAseq (Illumina). The data show that key bilin biosynthesis genes are coordinated with *Micromonas* phytochrome with a significant predawn peak, preceding the expression of photosynthesis-related genes. The expression of phytochrome protein and its subcellular localization under a diel showed redistribution from the cytosol to the nucleus throughout the day. These results are consistent with what we know for land plants, but also indicated that the light-dependent nuclear translocation of phytochrome predates the divergence of streptophytes and prasinophytes within the green lineage. Moreover, the *Micromonas* phytochrome displayed a previously undescribed light sensitivity, shifted to shorter wavelength relative to their land plant counterparts. This suggests that *Micromonas* phytochrome functions as a low light sensor better suited to aquatic environments where wavelengths are differently transmitted through water compared to terrestrial environments.

This research has important implications for understanding ancestral roles of phytochrome in plant development and influences adaptive on the circadian clock. Moreover, *Micromonas* provides a simplified model system to address the role played by phytochromes to light variations in changing oceans.

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